NONINVASIVE, TRANSCRANIAL AND LOCALIZED OPENING OF THE BLOOD-BRAIN BARRIER USING FOCUSED ULTRASOUND IN MICE

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Abstract—The feasibility of blood-brain barrier (BBB) opening in the hippocampus of wild-type mice using focused ultrasound (FUS) through the intact skull and skin was investigated. Needle hydrophone measurements through ex vivo skulls revealed minimal attenuation (~18% of the pressure amplitude), a well-focused beam pattern and minute focus displacement through the parietal bone. In experiments in vivo, the brains of three mice were sonicated transcranially. Pulsed ultrasound sonications at 1.5 MHz and acoustic pressures ranging from 0.8 to 2.7 MPa were used at 20% duty cycle. Before sonication, a bolus of 10 mL of an ultrasound contrast agents (Optison) was injected intravenously. Contrast-enhanced high-resolution magnetic resonance imaging (9.4 T) revealed BBB opening and allowed for the monitoring of the slow permeation of gadolinium in the hippocampus. The region of the brain where BBB opening occurred increased with the pressure amplitude. These findings thus demonstrated the feasibility of locally opening the BBB in mice using FUS through intact skull and skin and serve as the first step in determining and assessing feasibility of drug delivery to specific regions in the mouse brain using FUS. (E-mail: ek2191@columbia.edu) © 2006 World Federation for Ultrasound in Medicine & Biology.

Key Words: Ultrasound, Focused ultrasound, HIFU, Blood-brain barrier, Drug delivery, Magnetic resonance imaging.

INTRODUCTION

Many neurologic disorders remain intractable to treatment by therapeutic agents because of the brain’s natural defense, the blood-brain barrier (BBB). The BBB is a specialized vascular system consisting of endothelial cells with highly selective membranes connected together by tight junctions (Rubin et al. 1999). This system impedes entry of virtually all large molecules from blood to brain tissue, rendering otherwise potent neurologically active substances and drugs ineffective simply because they cannot be delivered to where they are most needed (Rubin et al. 1999). As a result, traversing the BBB has been the rate-limiting factor in brain drug delivery development (Pardridge 2005).

For a drug delivery system to be successful, transient, localized and noninvasive, targeting of a therapeutic agent is necessary. Most of the techniques currently being investigated do not offer all these attributes simultaneously. In “lipidization,” lipid groups are added to the polar ends of molecules to increase the permeability of the agent (Lieb et al. 1986; Fischer et al. 1998). This technique increases the permeability of the drug not only in the targeted region, but also the entire body, limiting drug dosage because of side effects. Other techniques under study are neurosurgically-based drug delivery methods, where drugs are invasively planted into a region by a needle (Blasberg et al. 1975; Fung et al. 1996). The drug spreads through diffusion and is localized to the targeted region, but diffusion does not allow for molecules to travel far from their point of release. In addition to this, invasive procedures traverse untargeted brain tissue, causing unnecessary damage. Other techniques utilize solvents mixed with drugs or adjuvants (pharmacologic agents) attached to drugs to disrupt the BBB through dilation and contraction of the blood vessels (Pardridge 2005). However, this disruption is not localized within the brain and the solvents and adjuvants used are potentially toxic. Finally, by understanding the structure and function of transporters endogenous to the cell membrane of the endothelial cells, intense chemical modifications of drugs may allow their passage through
these transporters (Pardridge 2005). This technique may constitute a delivery method specific to the brain, but it requires special attention to each type of drug molecule and a specific transport system, resulting in a time-consuming and costly process while still not being completely localized to the targeted region. The only technique that is a truly transient, local and noninvasive method to open the BBB is focused ultrasound (FUS) and microbubbles (Pardridge 2005).

Several studies have shown that FUS could locally disrupt the BBB while often causing undesired tissue damage (Bakay et al. 1956; Ballantine et al. 1960; Patrick et al. 1990; Vykhodtseva et al. 1995; Mesiwala et al. 2002; McDannold et al. 2004; Choi et al. 2005; Vykhodtseva et al. 1995; Mesiwala et al. 2002; McDannold et al. 2004; Choi et al. 2005; Kinoshita et al. 2006). However, through the introduction of microbubbles, FUS was found to open the BBB transiently (Hynynen et al. 2001). It was also found that the opening of the BBB could be monitored with contrast-enhanced MRI. The attractiveness in using microbubbles stems from the fact that it allows for reduction of the ultrasound intensity while containing the disruption within the vascular system, while leaving the neurons relatively unharmed (McDannold et al. 2005; Hynynen et al. 2005). There have also been preliminary studies for understanding the mechanism of the BBB opening, leading to indications of possible widening of the tight junctions or activation of various transport mechanisms (Sheikov et al. 2004). However, a complete understanding of the mechanism of BBB opening with FUS remains largely unknown. In addition, the safety and efficacy of the method remains to be demonstrated.

Although much progress has been made in FUS-induced BBB opening, before FUS can be implemented as a drug delivery method in the clinic, the mechanism of BBB opening must be understood while the safety and efficacy are determined. As a result, extensive experiments are required and an animal model needs to be carefully selected. Several factors in this choice need to be considered. First, an obvious problem with experiments of FUS in the brain is the strong amplitude and phase aberrations introduced by the skull. In humans, several studies have shown the possibility of correcting for these aberrations using adaptive techniques and these methods have been applied on animal skulls (Thomas et al. 1996; Hynynen et al. 1998). However, these techniques require complicated and specialized equipment (Pernot et al. 2003; Hynynen et al. 2004). In studies of BBB opening using FUS so far, animal experiments most often utilized a craniotomy to simplify experimental procedures. The decision to perform a craniotomy stems from the complexity of methods for correcting phase aberration. However, in mice the skull is much thinner than the ultrasound wavelength of 1 mm at 1.5 MHz and, therefore, should not cause significant aberrations. As a result, it may be possible to achieve ultrasound focusing in the murine brain without performing a craniotomy or applying aberration-correction techniques (Choi et al. 2005; Kinoshita et al. 2006). A second factor to consider is the sheer number of animals necessary in these experiments. Mice have the advantage of being more readily available to laboratories and easier to handle and care for than other animals. Finally and most importantly, the recent advent of transgenic engineering has allowed for the genetic expression of many neurologic diseases. However, most neurologic disease animal models are currently limited to mice, i.e., the only current animal models for Alzheimer’s and Huntington’s disease exist in mice. As a result, future studies of BBB opening require that methods of reproducible and accurate localized BBB opening with FUS be developed in mice.

The purpose of this paper is to address the technical problems associated with noninvasively opening the BBB in mice. First, ex vivo transcranial experiments are described; in these experiments, pressure fields are measured through ex vivo mouse skulls to evaluate the focusing quality of the ultrasound beam. A technique to target a region of the mouse brain accurately and precisely is also described. Second, in vivo experiments are described; these experiments investigate BBB opening using FUS with contrast-enhanced high-resolution magnetic resonance imaging (MRI). These studies demonstrate the feasibility of noninvasive, transcranial and localized drug-delivery in the brain of mice using FUS.

MATERIALS AND METHODS

Animals
A total of nine brown CB57-B16 type mice (Charles River Laboratories, Wilmington, MA, USA; mass: 23 to 28 g) were used in two sets of experiments. In the first set of experiments, six mice were used to obtain transcranial pressure measurements. The mice were sacrificed and their skulls wereexcised and degassed in saline. In the second set of experiments, four mice were used in sonication procedures. The mice were anesthetized with a mixture of ketamine (Fort Dodge Animal Health, Fort Dodge, IA, USA; concentration: 75 mg per kg of body mass) and xylazine (Ben Venue Laboratories, Bedford, OH, USA; concentration: 3.75 mg per kg of body mass). Before sonication, the hair on the top of the mouse heads was removed using an electric trimmer and a depilatory cream. After sonication, but before MRI scanning, the mice were switched to administration of isoflurane to simplify the long anesthesia procedure necessary for MRI scanning. During all imaging procedures, the vital signs of the mice were continuously monitored. Animals were euthanized immediately after MRI scanning. All procedures used on the mice were approved by the Co-
Ultrasound equipment

Ultrasound waves were generated by a single-element circular-aperture FUS transducer with a hole in its center for an imaging transducer (center frequency: 1.525 MHz; focal depth: 90 mm; outer radius: 30 mm; inner radius 11.2 mm). A single-element diagnostic transducer (center frequency: 7.5 MHz) with a focal length of 60 mm was positioned through the center hole of the FUS transducer so that the foci of the two transducers were properly aligned. A cone filled with degassed and distilled water was mounted on the transducer system. The water was contained in the cone by capping it with an ultrathin polyurethane membrane (Trojan; Church & Dwight Co., Inc., Princeton, NJ, USA), i.e., virtually transparent to the ultrasound beam. The FUS transducer was attached to a computer-controlled 3-D positioning system (Velmex Inc., Lachine, QC, Canada). It was also driven by a function generator (Agilent Technologies, Palo Alto, CA, USA) through a 50-dB power amplifier (ENI Inc., Rochester, NY, USA), while the diagnostic transducer was driven by a pulser-receiver system (Panametrics, Waltham, MA, USA) connected to a digitizer (Gage Applied Technologies, Inc., Lachine, QC, Canada). A 3-D raster-scan (lateral step size: 0.2 mm; axial step size: 1.0 mm) of the FUS transducer was performed in degassed water with a needle hydrophone (Precision Acoustics Ltd., Dorchester, Dorset, UK; needle diameter: 0.2 mm). The dimensions of the beam were measured to have a lateral and axial full-width at half-maximum (FWHM) intensity of approximately 1.32 and 13.0 mm, respectively.

Ex vivo experiments

The effect of the mouse skull on the ultrasound beam propagation using a single-element transducer was investigated using six excised mouse skulls. Each skull was separately placed and held stationary in a tank filled with degassed water. The transducer system was submerged in the water tank and held stationary above the excised skull, with its focus placed 3 mm beneath the top of the skull. The needle hydrophone was (Precision Acoustics Ltd., Dorchester, Dorset, UK; needle diameter: 0.2 mm). The dimensions of the beam were measured to have a lateral and axial full-width at half-maximum (FWHM) intensity of approximately 1.32 and 13.0 mm, respectively.

In vivo experiments

Experimental set-up. Four mice were anesthetized and placed prone on a polyurethane bed (Fig. 1). A water bath, the bottom of which consisted of an ultrathin acoustically and optically transparent plastic layer (Saran; SC Johnson, Racine, WI, USA), was filled with degassed and distilled water and suspended over the anesthetized mouse’s head. Ultrasound gel was used further to reduce any remaining impedance mismatches between the thin plastic layer and the mouse skin. Finally, the FUS transducer was placed in the water bath with its beam axis perpendicular to the surface of the skull.

Targeting procedure. The focus of the transducer was positioned inside the mouse brain using a grid positioning method. In this method, the sutures of the mouse skull seen through the skin were used as anatomic landmarks for targeting purposes. The location of the hippocampi were assumed relative to the sutures based on the mouse brain and known skull anatomy (Fig. 2a, b). A grid consisting of three equally spaced 0.3-mm thin metal bars was placed in the water bath on top of the skull and in alignment with these sutures. The first bar was aligned parallel and along the sagittal suture, and the
second bar was attached perpendicularly to the first bar and in alignment with the suture between the parietal and interparietal bone. In CB57-b16 type mice, these were the sutures that could be clearly seen through the skin. The third bar was placed 4 mm away from and parallel to the second bar. Using this grid positioning system, one of the hippocampi was reproducibly targeted when assumed to be at mid-distance between the parallel bars and 2 mm away from the center bar (Fig. 2b). A C-scan of the grid using the diagnostic transducer was generated and the location of the hippocampus was identified relative to this grid (Fig. 2c). The focus of the FUS transducer was placed 3 mm beneath the top of the skull by measuring the distance with the diagnostic transducer. Using the grid positioning method and depth calculations, precise, accurate and reproducible targeting of the hippocampus of the mouse brain was performed.

To determine the accuracy of this positioning system, a separate set of preliminary experiments was performed. It was determined that the intended target was within 0.5 mm of the actual focus. Considering the 1.32-mm lateral FWHM beam used in these experiments, the grid positioning method was sufficiently precise to have the FUS beam consistently overlap the hippocampus of the murine brain.

Opening of the BBB. Experiments to determine whether the BBB could be opened using the previously described methods were performed. A bolus of 10 μL (approximately 0.4 mL/kg) of an ultrasound contrast agent (Optison) that contained microbubbles (mean diameter: 3.0 to 4.5 μm; concentration: 5.0 to 8.0 × 10^8 bubbles per mL) was injected into the right femoral vein of the mouse approximately 15 min before sonication (Table 1). Pulsed-wave FUS (burst rate: 10 Hz; burst duration: 20 ms; duty cycle: 20%; acoustic pressures at the focus: 2.0, 2.5 and 2.7 MPa) was then applied in a series of five shots lasting 30 s each with a 30-s delay between each shot. The FUS sonication procedure was performed once in each mouse brain. The acoustic pressure values were obtained from the values found in degassed water and corrected using the attenuation values of the skull. The sonications were focused at the left hippocampus of the mouse brain, and the right hippocampus was not targeted and acted as the control. The pressure values 2.0, 2.5 and 2.7 MPa were selected after a preliminary study that determined the threshold of BBB opening to be around 2.5 MPa, given the aforementioned set-up parameters.

To investigate the effect of the 15-min delay between the

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**Table 1. Experimental timeline for the in vivo study**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Procedure</th>
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<tbody>
<tr>
<td>0</td>
<td>Administration of anesthesia</td>
</tr>
<tr>
<td>15</td>
<td>Injection of Optison</td>
</tr>
<tr>
<td>30</td>
<td>Sonication</td>
</tr>
<tr>
<td>45</td>
<td>Start of T1 MRI scan acquisition (NEX: 15)</td>
</tr>
<tr>
<td>60</td>
<td>Injection of gadolinium</td>
</tr>
<tr>
<td>60</td>
<td>Start of T1 MRI scan acquisition (NEX: 10)</td>
</tr>
<tr>
<td>70</td>
<td>Start of T2 MRI scan acquisition (NEX: 10)</td>
</tr>
<tr>
<td>80</td>
<td>Start of T1 MRI scan acquisition (NEX: 15)</td>
</tr>
<tr>
<td>95</td>
<td>Start of T2 MRI scan acquisition (NEX: 15)</td>
</tr>
<tr>
<td>110</td>
<td>Start of T1 MRI scan acquisition (NEX: 45)</td>
</tr>
<tr>
<td>155</td>
<td>Start of T2 MRI scan acquisition (NEX: 45)</td>
</tr>
</tbody>
</table>
between Optison injection and sonication, the presence of Optison in the bloodstream after such a time delay was verified. A separate study was performed, in which two mice were injected with Optison intravenously (IV) and their left ventricles were imaged with a high-resolution ultrasound system (Visual Sonics; Toronto, Ontario, Canada; frequency: 35 MHz). The echocardiograms indicated that Optison was still present at least 30 min after IV injection, and its concentration in the bloodstream was steadily decreasing over time. Although Optison was still present beyond 30 min, no further monitoring was performed. Finally, to further investigate the importance of the concentration of Optison (and, thus, timing of sonication post injection) on the BBB opening and the pressure amplitude used, the previously described sonication procedures were performed at 1-min post injection of 25 μL of Optison at the lower pressure amplitude of 0.8 MPa.

**MRI.** T1- and T2-weighted MRI scans were obtained using a 9.4 T system (Bruker Medical; Boston, MA, USA) (Table 1). The mice were placed in a plastic tube with a 3.8-cm diameter birdcage coil attached and were inserted vertically into the magnet. Approximately 15 min after sonication, but before MRI contrast agent injection, a T1-weighted spin-echo MRI scan was obtained (repetition time/echo time [TR/TE]: 246.1 ms/10 ms; bandwidth [BW]: 50,505.1 Hz; matrix size: 256 × 256; field of view [FOV]: 1.92 × 1.92 cm; slice thickness: 0.6 mm; number of excitations [NEX]: 10, 15 and 45). These images were used to determine whether FUS had caused any tissue damage. Once the first scan was completed, 0.5 mL of MRI contrast agent gadolinium (Omniscan; Amersham Health, AS Oslo, Norway) was administered intraperitoneally via a catheter to depict BBB opening (Barzó et al. 1996). Intraperitoneal injection allowed for the slow uptake of the MRI contrast agent into the bloodstream (Moreno et al. 2006). After injection of the MRI contrast agent, a series of six alternating T1-weighted and T2-weighted fast spin-echo image scans (TR/TE: 4000 ms/9.2 ms; rapid acquisition with relaxation enhancement: 16; FOV: 1.92 × 1.92 cm; matrix size: 256 × 256; number of slices: 10; slice gap: 0.1 mm; NEX: 10, 15 and 45) were performed after each mouse.

**MRI analysis.** Contrast-enhanced behavior was followed for a period of 140 min after injection of gadolinium, to assess the time course of BBB opening. For each MRI scan, a 15 × 15 pixel area of a nonsonicated homogeneous brain region was averaged. The entire MRI scan was then divided by this averaged value. The left (FUS-targeted) and right (control) hippocampi were compared in each mouse and any pixel intensity value above 2.5 standard deviations was determined to be a contrast-enhanced region, revealing BBB opening. Thresholding by 2.5 standard deviations was used because it provided a significantly clear differentiation between unaffected and BBB-opened regions in all mouse experiments. The approximate area of the BBB opening region was then calculated by counting the pixels above the threshold.

**RESULTS**

**Ex vivo experiments**

Ultrasound through the parietal bones on the left and right halves of the sagittal suture provided the least amount of attenuation (~18.1% of the pressure amplitude; Fig. 3) when compared with other regions of the skull. The FWHM was equal to 1.33 mm through the...
skull and 1.32 mm when no skull was present. The change in the location of the focus as a result of skull aberration was lower than the resolution allowed by the needle hydrophone (needle diameter: 0.2 mm). The pressure field measurements revealed no significant distortion of the ultrasound beam shape or focus location (Fig. 3), allowing for a single-element FUS transducer to be used in opening of the BBB through an intact skull.

**In vivo experiments**

In most cases, no visible damage was detected on the T1 MRI scans obtained after sonication and before MRI contrast agent injection. Gadolinium was then injected to determine whether the BBB was opened (Barzó et al. 1996; Hynynen et al. 2001). After sonication at a peak pressure amplitude of 2.0 MPa and injection with MRI contrast agent no contrast enhancement was observed (Fig. 4). At 2.5 and 2.7 MPa, MRI contrast agent injection depicted BBB opening (Figs. 5 and 6). A temporal analysis of this opening was made over a 140-min period, revealing leakage of the MRI contrast agent from the posterior cerebral artery (PCA) or its adjacent arterioles and capillaries to the surrounding brain tissue. Sonication at a peak pressure amplitude of 2.5 MPa allowed for a highly localized opening of the BBB near the PCA (Fig. 5a-c). Figure 6a-c shows how, after sonication at a peak pressure amplitude of 2.7 MPa (as in the 2.5 Mpa), the gadolinium first appears in the PCA only (10 min post-injection), then slowly permeates throughout the surrounding regions (35 min post-injection), eventually encompassing the entire left hippocampus (95 min post-injection). On T2 MRI scans, BBB opening resulted in a decrease in pixel intensity and the area affected was in good agreement with what was seen on the T1 MRI scans (Fig. 5d, e and Fig. 6d, e). Over the time studied, the area of contrast enhancement increased (Table 2). The extent of the region where BBB opening occurred also varied with ultrasound pressure amplitudes (Figs. 5 and 6). At the pressure amplitude of 2.0 MPa, there was no opening of the BBB, and the area of BBB opening increased with the pressure amplitude above 2.5 MPa. Finally, when the timing of Optison injection was changed from 15 min to 1 min before FUS sonication and the injection was increased to 25 µL, opening of the BBB occurred at the far lower threshold of 0.8 MPa (Fig. 7).

**DISCUSSION**

This study demonstrated accurate and precise opening of the BBB through the intact skull and skin in mice with a single-element FUS transducer. MRI scans at 9.4 T and gadolinium injected intraperitoneally allowed for a highly detailed spatial and temporal analysis of the BBB opening. There was minimal attenuation and distortion of the ultrasound beam, resulting in a well-controlled beam pattern through the parietal bone of the skull, as shown from the ex vivo experiments (Fig. 3). Other regions of the skull, however, presented greater difficulties in obtaining a well-formed ultrasound beam in the brain as a result of the thicker layers of skull in those regions (i.e., the interparietal bone; Fig. 2a) as established by micro-computed tomography (CT) scans (Choi et al. 2005). Further investigations on the entire skull need to be performed if studies of FUS in brain regions other than through the parietal bones are to be made. In this study, we were not interested in all skull regions because the main focus was the treatment of neurodegenerative diseases that affect the hippocampus. Precise targeting of the hippocampus was accomplished without any need for an
MRI targeting device such as may be used in other studies (Hynynen et al. 2001) and with a relatively inexpensive and precise grid positioning method that utilized the sutures of the skull as anatomical landmarks. It should be noted, however, that the sutures could not be clearly seen through the skin of every type of mouse and, in the CB57-b16 type mouse, only the sagittal and lambdoid sutures could be clearly distinguished. The accuracy and precision of the targeting techniques described were concluded to be more than sufficient for the purpose of these experiments and consistently opened the BBB in the regions-of-interest (Figs. 5 and 6).

Fig. 5. T1 MRI scans of horizontal slices of a single mouse brain approximately 3 mm beneath the top of the mouse skull. Images were obtained after sonication with a pressure amplitude of 2.5 MPa. The ultrasound beam was focused onto the left hippocampus. The right hippocampus was not targeted and acted as a control. (a) 10 min after gadolinium injection, a subtle increase in contrast enhancement indicates gadolinium beginning to leak through the posterior cerebral artery near the hippocampus. (b) 35 min after gadolinium injection, contrast enhancement increased significantly, indicating permeation of gadolinium through the region. (c) 95 min after gadolinium injection, gadolinium has spread to a localized region around the posterior cerebral artery. T2 MRI scans were obtained (d) 20 min and (e) 50 min after gadolinium injection, revealing suppressed signal (appearing at low pixel intensity) where gadolinium is released. This corresponds to the regions of contrast enhancement seen on the T1 MRI scans in (b) and (c).

Most pressure amplitudes used were higher than those used in other studies (Hynynen et al. 2001; McDannold et al. 2004). This is because of the 15-min delay in sonication after IV injection of Optison, resulting in a lower concentration of Optison present in the bloodstream during the sonication. This delay in injection was used to simplify the experimental procedures by not requiring IV catheterization, or injection into a smaller vein, which can be difficult and expensive procedures to perform in mice. This delay was not a significant concern because the purpose of this study was to determine the feasibility and reproducibility of BBB opening, as shown in Figs. 5 and 6. When the sonication was performed 1 min after injection of Optison, the BBB opening occurred at a lower pressure threshold of 0.8 MPa (Fig. 7). Earlier injection and, thus, higher Optison concentration in the bloodstream allowed for a reduced pressure amplitude necessary for BBB opening. This reduction in pressure amplitude further decreases the probability for thermal damage or other forms of damage at or around the BBB-opened brain region. Future studies will be concerned with characterizing the dependence on Optison concentration, as well as with designing the optimized experimental protocol and FUS parameters necessary to deliver both safe and efficient BBB opening.
Mice were selected as the animals for our study, mainly because many neurologic disease models are available for mice while they currently do not exist in any other animals. In addition to this, mice can be obtained easily in large numbers and are easy to manage. This is necessary for future BBB opening studies, especially when trying to optimize the ultrasound parameters. As a result, we addressed the technical problems associated with FUS studies on the murine brain, successfully showing the feasibility and monitoring of BBB opening using FUS and high-resolution MRI, respectively. There are some concerns when dealing with ultrasound at 1.525 MHz applied to the murine brain because of its relatively small size when compared with the ultrasound focus size. It may be difficult to completely localize the ultrasound energy to a specific location without interfering with an untargeted part of the brain, such as the ventricles or the skull. In addition to this, there are concerns with the ultrasound beam propagation near the ear canal system. Because the beam is cigar-shaped, with its longest dimension in the axial direction, the beam shape of high ultrasound intensities in these regions may be unpredictable. Regardless of these issues, there are still several

Table 2. Area of contrast enhancement for different pressure amplitudes and times. The area of contrast enhancement increased with increasing amplitude. Also, once the BBB was opened, IP injection of gadolinium allowed for the eventual increase in area of contrast enhancement.

<table>
<thead>
<tr>
<th>Pressure amplitude (MPa)</th>
<th>Area of contrast enhancement (mm²)</th>
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<tr>
<td></td>
<td>At time = 10 min</td>
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<tr>
<td>2.0</td>
<td>0</td>
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<tr>
<td>2.5</td>
<td>0</td>
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<tr>
<td>2.7</td>
<td>33.5</td>
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Fig. 6. T1 MRI scans of horizontal slices of a single mouse brain approximately 3 mm beneath the top of the mouse skull. Images were obtained after sonication with a pressure amplitude of 2.7 MPa. The ultrasound beam was focused onto the left hippocampus. The right hippocampus was not targeted and acted as a control. (a) 10 min after gadolinium injection, gadolinium began to leak through the posterior cerebral artery near the hippocampus. (b) 35 min after gadolinium injection, permeation through the region increased until (c) 95 min after gadolinium injection, when gadolinium had permeated the entire hippocampus. T2 MRI scans were obtained (d) 20 min and (e) 50 min after gadolinium injection, revealing suppressed signal (appearing at low pixel intensity) where gadolinium is released. This corresponds to the regions of contrast enhancement seen on the T1 MRI scans in (b) and (c).
areas of the brain where the FUS can be properly used and localized without significantly affecting untargeted regions of the brain.

More important, aside from the feasibility of the BBB opening is the demonstration of the utility of high-resolution MRI (9.4 T) and intraperitoneal injection of MRI contrast agents to attain highly detailed spatial and temporal information of the BBB opening. Reported here, for the first time to our knowledge, Figs. 4 through 6 provide examples of monitoring slow contrast diffusion through the FUS-induced BBB opening. At high resolution and utilizing the slow diffusion of a molecule that would otherwise not traverse the BBB, a substantially more detailed temporal and spatial analysis of the region enhanced by the MRI could be obtained, including accurate measurement of the region being affected by the BBB opening (Table 2). With a 9.4-T magnetic field, the study of the mechanism of the opening is facilitated. For example, the vessel density and size appear to play a significant role in the way that the MRI contrast agent permeates the BBB. Figure 6 shows how the MRI contrast agent first appears in the PCA or the region around the hippocampus, where the BBB was opened at 2.7 MPa using the same sonication parameters without Optison injection, revealed no structural damage (Fig. 8). Future studies will also test for more subtle forms of damage, such as apoptosis and necrosis. A long-term temporal analysis will also be performed to determine how long the BBB in mice stays open. Finally, the techniques described may eventually be used to test for molecular delivery to the hippocampus, or other deep-seated subcortical brain regions, of neurodegenerative-disease–affected animals.

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Fig. 8. Two histologic horizontal sections (crystal violet stain) of the mouse left hippocampus at approximately (a) 1.9 mm and (b) 2.0 mm from the top of the skull, with a pressure amplitude of 2.7 MPa and no Optison, revealing no structural damage.